traverse (OA §§1-6). The traversal is on the ground that because the DNA of I encodes the protein of II, it is not unduly burdensome to search both groups.

1.2. A substitute declaration is enclosed (OA §9). It is in two overlapping parts: (1) signed by inventors Kappeller, van den Brink, Rahbek-Nielsen, and Budtz, and (2) signed by inventors Farah, van den Brink, Rahbek-Nielsen and Budtz.

1.3. Substitute drawings are enclosed (OA §10). The PTO-948 objected only to Figs. 1-5, not to 6-10. We have nonetheless also submitted a substitute Fig. 6 as its curve appeared "staggered".

2. Prior Art Issues

2.1. Claims 49-51, 5, 7 and 10 stand rejected as anticipated by Nomura et al. Nomura disclosed production of a fusion protein comprising rat apolipoprotein E and "Mucor rennin" in S. cerevisiae. The Examiner observes that Mucor is "non-bovine".

Claim 49 has been amended to limit the chymosin (rennin) to one of a mammalian species of suborder Tylopoda, as supported by page 6, line 15. The other rejected claims are dependent on 49. Since Mucor is not even mammalian, let alone Tylopoda, the anticipation rejection is overcome.

2.2. Claims 2, 5-8, 10-11 and 49-51 are rejected as obvious over Houen (who discloses porcine chymosin) in view of Ward (who discloses a yeast expression vector for bovine prechymosin). Similarly, claims 2-3, 5-8, 10-11 and 49-51 are rejected as obvious over Pungercar (lamb prepro chymosin) in view of Ward.

The limitation to suborder Tylopoda overcomes both rejections.

Applicants have demonstrated that camel chymosin has a specific kappa casein hydrolysing activity superior to that of bovine chymosin (P4, L18-20). This can be expressed in the form of a "C/P ratio", which is the ratio of that specific activity to the nonspecific proteolytic activity. As shown in table 5.1,

the C/P ratio of camel chymosin is 7.00, while that of bovine chymosin is only 1.00. Moreover, the overall milk clotting activity of camel chymosin is 170% that of bovine chymosin.

Camels belong to the suborder Tylopoda, whereas bovines and ovines belong to the suborder Ruminanta, and pigs to the suborder Suina. (These are the three suborders of the Order Artiodactyla.) All living tylopods are members of the family Camelidae. This family includes camels, llamas, alpacas, vicugnas and guanacos.

While we do not have sequence data for tylopod chymosins other than Camelus dromedarius, we can form a general idea of the relatedness of tylopod proteins (1) to each other and (2) to cognates in other mammalian taxons, by reviewing the results of a BLAST search (with low complexity filtering inactivated) on the hemoglobin alpha chain of C. dromedarius:

Species	E value	% identity
Lama guanico (guanaco)	1e-75	97%
Lama vicugna (vicugna)	2e-75	97%
Lama glama (llama)	1e-74	96%
Colobus badius (red colobus)	1e-67	87%
Hippopotamus amphibius	4e-67	86%
Ceratotherium simium (white rhino)	3e-66	86%
Rhinoceros unicornis	1e-65	85%
Sus scrofa (pig)	1e-65	85%
Bos taurus	2e-64	85%
Elephas maximus	3e-63	81%
Ovis aries	3e-63	82%

Colobus badius was the highest scoring non-tylopod. Hippopotamus amphibius was the highest scoring non-tylopod member of the Order Artiodactyla.

If we compare camel chymosin to the chymosins of other

species, we obtain

% similarity % **identity**	Camel	Cow	*B. p.*	Water buffalo	Sheep	Pig
Camel	100 **100**	89 **84**	80 **75**	84 **80**	88 **84**	85 **81**
Cow (B. bovis)		100 **100**	90 **90**	94 **94**	96 **95**	86 **80**
Bos Primigensis			100 **100**	92 **92**	86 **84**	79 **73**
Waterbuffalo				100 **100**	91 **90**	77 **77**
Sheep					100 **100**	85 **80**
Pig						100 **100**

The identity and similarity were calculated by use of align X, which is part of the vector NTi package. The align X program implements the clustal W algorithm of Thompson, et al., Nucleic Acids Res., 22(22):4673-80 (1994).

Camel chymosin is less than 90% identical to the chymosin of the non-tylopod hoofed mammals noted. The same was true of the relationship of camel alpha globin to non-tylopod alpha globins. In contrast, camel alpha globin was at least 95% identical to the alpha globins of its fellow tylopods guanaco, vicugna and llama.

In view of the known and expected sequence differences between the tylopod (prepro) chymosins and the non-tylopod (e.g., bovine, sheep, pig) chymosins, and the known superiority of camel chymosin over bovine chymosin, we believe that the obviousness rejection should be withdrawn now that the claims are limited to tylopods.

3. Description/Enablement Issues

1. The Examiner concedes description and enablement for

camel chymosin, but not for all "non-bovine chymosins". She takes a parallel position with respect to prochymosin and preprochymosin.

Under the "description" rejection (OA §26), the issue is whether camel chymosin is representative of the claimed genus, which in turn depends on whether the other members of the genus would be expected to share the relevant properties of camel chymosin. Under the "enablement" rejection (OA §27), the issue is whether it would require undue experimentation to isolate other chymosins within the claimed genus and determine whether they had the claimed properties.

In the discussion of the description requirement, the Examiner brings up two points: (1) whether Applicant has adequately identified the "critical structural elements" required for chymosin activity and milk clotting activity, and (2) whether the elements that permit camel chymosin to outperform bovine chymosin and known.

With respect to point 1, the specification identifies the active site of chymosin at page 1, lines 24-35. The noted "DTG" is at positions 92-94 of camel chymosin.

Moreover, the present claims are not directed to non-naturally occurring mutants with a particular minimum sequence identity to camel chymosin. Rather, they are directed to expression of a naturally occurring, non-bovine chymosin, prochymosin, or preprochymosin. These proteins possess chymosin activity by definition.

With regard to the structural features giving camel chymosin superiority over bovine chymosin, the skilled worker would naturally consider where camel chymosin diverges from bovine chymosin, especially within those regions which, looking at all the known chymosins (which include water buffalo, cat, sheep, pig, rat, mouflon, marmoset and goat) appear likely, based on clustering of conserved residues, to be responsible for chymosin activity.

Again, we are not concerned with non-naturally occurring

chymosins, but rather with identifying naturally occurring ones which share camel chymosin's superiority. Plainly, one would start by testing the chymosins most closely related to camel chymosin, i.e., llama, alpaca, etc. The Bork article, cited by the Examiner, says that the prediction of functional features by homology is 90% accurate (see Table 1, third-to-last item).

The Examiner cites articles to show that small changes can drastically change the activity of a polypeptide. We do not dispute that this is the case. However, it is equally well known that, in general, the greater the degree of sequence identity/similarity between two proteins, the more likely they are to have the same activity. Moreover, the process of sequence alignment not only permits calculation of the overall sequence identity/similarity between to proteins, but also the identification of conserved and unconserved positions, and of the substitutions tolerated at particular positions.

In the case of Witkowski, the researchers deliberately modified a cysteine known to be the active site of the beta-ketoacyl synthase, so it is hardly surprising that such activity was lost. (The real surprise was that malonyl decarboxylase activity appeared). It is important to distinguish between deliberate attacks on functional site residues, and modifications intended to preserve activity.

In the enablement rejection, the examiner asserts that there are an "extremely large number of unknown pre-prochymosins, prochymosins, and chymosins encompassed by the claims". Given the present limitation to tylopods, that is certainly true no longer. The Tylopoda are a rather small suborder. It has three genera: Camelus (with two species), Lama (with three), and Vicugna (with one).

It is respectfully urged that in view of the limitation of claim 49 to Tylopoda, the rejections stated in OA §§26, 27 should be withdrawn.

2. Claims 9 and 12 which refer to specific deposits (CBS 108915 and CBS 108916) of Aspergillus niger strains have been

rejected (OA §29) because it is not clear on the record that the deposit requirements of 37 CFR §§1.801-1.809 have been complied with.

"CBS" -- more properly, "Centraalbureau voor Schimmelcultures" -- is an International Depository Authority under the Budapest Treaty, and the deposits in question were made under the Budapest Treaty. Indeed, page 22, lines 22-25 state

> A sample of the strains #21 and #28 were deposited under the Budapest Treaty with the Centraalbureau voor Schimmelcultures (CBS), Oosterstraat 1, P.O. Box 273, 3740 AG Baarn, The Netherlands, on 13 June 2000 under the accession Nos. 108915 and 108916, respectively.

Hence, it appears that, to complete compliance, all that is necessary is a statement concerning public availability.

Counsel hereby states, on behalf of applicants, that CBS 108915 and BCS 108916 were deposited under the Budapest Treaty, and will be irrevocably and without restriction or condition released to the public upon the issuance of a patent on this application.

4. Definiteness Issues (OA §§14-24) and Claim Objections (OA §§11-13)

We agree with the Examiner that the paragraphing of claim 49 is confusing. "(I)" should refer to the coding sequence, not to the properties of the protein. We have corrected this.

The term "non-bovine" was intended to apply to the "prochymosin" and the "chymosin", as well as to the immediately adjacent term "pre-prochymosin". We have taken out this term as unnecessary in view of the limitation to Tylopoda.

Note that claim 49 now recites "a pre-prochymosin, prochymosin, or chymosin, of a mammal of the suborder Tylopoda". It is clear as a result of the underlined comma that the "of...Tylopoda" phrase applies to each of the three objets which precede it, not just to the nearest one.

OA §17 (claim 51)

See our comment on "non-bovine" in claim 49

OA §18 (claim 2)

Claim 2 has been cancelled since (1) base claim 49 has been limited to Tylopoda, and (2) all living Tylopoda are Camelidae (camelids). However, we have added a claim (52) reciting the genus Camelus (P6, L10).

OA §19 (claim 4)

We have amended claim 4 to require that the encoded (pre)(pro)chymosin be a Camelus dromedaries protein. The DNA need not be isolated from that organism or even be identical to the native gene, i.e., silent substitutions are allowed.

OA §20 (claim 8)

We have replaced "expression vector" with "DNA construct", consistent with base claims 49 and 50.

OA §21 (claim 8)

We have deleted "as described in Ward et al. 1990".

OA §22 (claim 8)

We do not agree with the Examiner's position that a "coding sequence" is merely a "graphical representation". It is only the string of letters which is the graphical/textual representation of the coding sequence, the latter being a physically identifiable chemical moiety within a DNA molecule (the "DNA construct"). Nor do we agree that the term "polynucleotide" would be clearer. The whole DNA construct is a polynucleotide, but the coding sequence is merely a portion of that polynucleotide. The term "polynucleotide" could be interpreted by the art as referring only to a unitary molecule.

The term "coding sequence" appears as such in the claims of 1235 U.S. patents issued since 1976, so it can hardly be

considered indefinite.

Nonetheless, we have amended claim 8 to clarify the "derived" language.

OA §24 (claim 15)

Claim 15 has been amended to depend from claim 51.

OA §13 (claim 51)

The Examiner says that claim 51 is objected to "because of the recitation of "if said expressible protein is a fusion protein, cleaving it to release said protein of interest". The objection is traversed because it does not explain why this recitation is objectionable. If the problem is with the use of an "if" clause, we must point out that many U.S. patents have claims with such clauses, although they are difficult to identify by a USPTO database search because the word "if" is a "stop word".

Base claim 49 plainly recites that the coding sequence may encode "(b) a fusion comprising a core protein which is such a pre-prochymosin, prochymosin or chymosin, and cleavable to release said core protein".

Claim 51 recites the cleaving step, albeit conditionally, because its "protein of interest" is not the fusion protein per se, but rather the Tylopoda core protein within that fusion protein.

OA §11 (claim 7)

We have inserted the comma after "prochymosin", and additionally recite just "or fusion protein" instead of "or a fusion protein thereof", as the relationship of the fusion protein to the first three proteins is explained in base claim 49.

OA §12 (claim 13)

We agree with the Examiner that the only difference must be

in the polynucleotide encoding the protein. However, the point of the claim was to limit the difference to the coding sequence, as otherwise the difference could be attributable to another feature of the vector.

We have rewritten claim 13 to restate this with a minimum of verbiage.

5. Miscellaneous

We have amended claim 49 to recite an "isolated or non-naturally occurring" DNA construct to avoid any possibility that the claim would read on a mammalian chromosome.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

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Enclosures
-substitute declaration
-substitute drawings

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Drawings

Substitute drawings (Figs. 1-6) are being filed.

In the claims:

Claims 4, 7, 8, 13, 15, 49, and 51 have been amended as follows:

4 (twice amended). A method according to claim 51 wherein the [coding sequence for] pre-prochymosin, prochymosin [and] or chymosin is [isolated or derived from] a *Camelus dromedaries* protein.

7. (twice amended). A method according to claim 51 wherein the pre-prochymosin, prochymosin, [or] chymosin, or [a] fusion protein [thereof,] is secreted over the host cell membrane.

8. (twice amended). A method according to claim 51 wherein the DNA differs [expression vector is derived] from pGAMpR [as described in Ward et al., 1990 by substituting] solely in that the coding sequence is different [of that vector for bovine prochymosin with a coding sequence for the non-bovine pre-prochymosin, prochymosin or chymosin].

13 (twice amended). A method according to claim 51 wherein the yield of pre-prochymosin, prochymosin or chymosin milk clotting activity is at least 25 % higher than the yield of bovine pre-prochymosin, bovine prochymosin or bovine chymosin milk clotting activity obtained when using, under identical production conditions, an [the same] expression vector which differs only with respect to its[, but with a] coding sequence [for bovine pre-prochymosin, prochymosin or chymosin in place of the coding sequence for the non-bovine pre-prochymosin, prochymosin or chymosin].

15 (amended). A method according to claim [1] 51 wherein the host cell is a cell expressing a deglycosylating enzyme

49. (amended). [A] An isolated or non-naturally occurring DNA construct, the nucleic acid sequence of which comprises (I)

a coding sequence coding for an expressible protein which is [(I)] (a) a [non-bovine] pre-prochymosin, prochymosin, or chymosin of a mammal of the suborder Tylopoda or (b) a fusion protein comprising a core protein which is such a pre-prochymosin, prochymosin or chymosin, and cleavable to release said core protein; and

(II) appropriate expression signals, operably linked to said coding sequence, permitting the protein to be expressed in a host cell.

51 (amended). A method of producing a Tylopoda protein of interest selected from the group consisting of [non-bovine] pre-prochymosin, prochymosin, and chymosin which comprises

providing a host cell according to claim 50,

cultivating said host cell under conditions where said expressible protein is expressed,

if said expressible protein is a fusion protein, cleaving it to release said protein of interest, and

harvesting the protein of interest.

Claims 2, 3, and 35-40 have been cancelled.

Claim 52 has been added.

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